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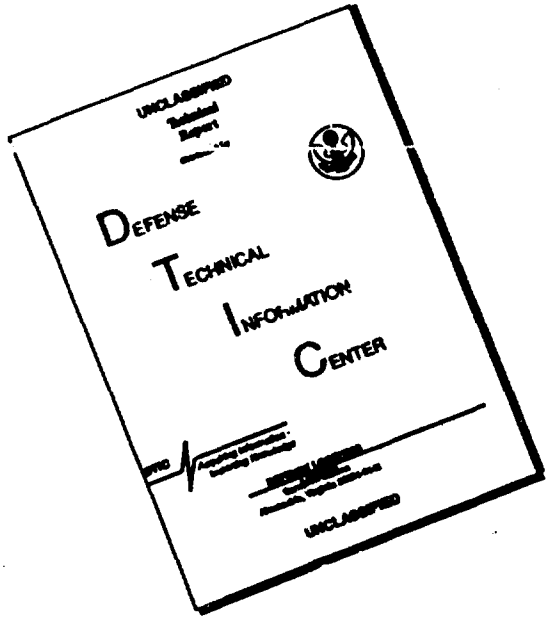

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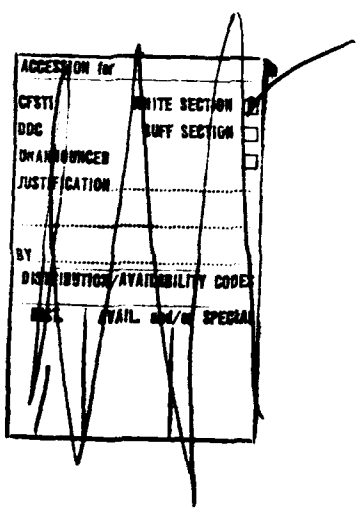

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CONCERNING THE MAIN PROPERTIES OF STRAINS OF P. PESTIS ISOLATED IN 1962 IN ARMENIA

[Following is the translation of an article by A. A. Vartanyan, R. S. Mikhaylova, M. L. Sukiasyan and M. T. Shekhikyan, Armenian Antiplague Station and the Scientific-Research Antiplague Institute for Kavkas and Zakavkaz, published in the Russian-language periodical Trudy Armyanskoy Protivochumnoy Stantsii (Trudy of the Armenian Antiplague Station), No 3, 1964, pages 45-49. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

From July through August 1962 in the territory of the northwest outskirts of Zangezur in the Armenian SSR, a plague epizootic was observed among common voles. During the stated period the laboratory of the Sisian Epidotryad investigated 2347 specimens of common voles, 484 social voles, 197 snow voles, 22 water voles, 13 bush voles, 435 forest mice, 209 house mice, 75 Zakavkazskiy hamsters, 62 forest dormouses, 4 Persian gerbils, and 1 slepushonka [Meaning unknown, probably related to SLEPYSHI - Spalacidae (a family of rodents)].

All told during the period of observation 47 strains of P. pestis were isolated; of these 14 were from common voles and 33 from fleas collected in the nests of these voles (Ctenophthalmus wladimiri - 27 strains, Ceratophyllus caspius - 3, Frontopsylla elata caucasica - 2, Amphipsylla rossica - 1).

The investigation of rodents of other species, captured in the zone of the epizootic and adjoining regions, produced a negative result in all cases.

In the process of the investigation on the territory where the epizootic had taken place, not one rodent was detected which had died from plague. The cultures of the plague microbe were isolated only from voles which had been captured alive. Apparently the infectious process in common voles took place mainly in a benign manner. This assumption is supported to a certain degree by the data of laboratory investigations. Thus, for example, 6 strains were obtained from animals, in whose organs upon autopsy there were noted only the necrotic changes which are characteristic for a lingering course of the disease. Besides this, in 6 rodents from which cultures of the plague microbe were isolated, apparent pathologoanatomical changes were generally absent.

It is necessary to note that in a whole number of cases the direct inoculation of the organs of rodents and fleas on nutrient media did not expose the presence of the causative agent in them and cultures of the plague microbe were obtained only due to a biological test animal.

In the present report the materials are presented which characterize the main properties of the 44 strains of P. pestis which were isolated during the epizootic.

At the time of isolation all the strains were found in the R-form. During the course of a 2--3 week period of observation following isolation, the phenomena of dissociation and the signs of affection by bacteriophage were not noted in the cultures (an exception was strain 367, obtained from a white mouse, infected by a group of Ct. wladimiri fleas, in which the growth of the structure of the colonies became very untypical on the second day). In the majority of the strains the form of the colonies was the same and was characterized by a dark brown, raised, fine grained center with a smooth, pale, irregular edge, and a lacy peripheral zone. Upon aging the colonies lost the peripheral zone, the center became more compact and the intensity of pigmentation increased.

Growth on broth was typical -- the broth was clear, there was a porous precipitate and a parietal granular or flaky suspension. While the broth cultures were maintained in the incubator, a delicate film, which split easily upon shaking, developed. During the study of the strains in the first generations, turbidity of the broth was not noted.

In smears from the organs of voles and from biotest animals the microbe was in the form of a polymorphic gram-negative bacillus with bipolar staining. Mobility of the bacterial cells was absent (checking was done on semiliquid agar).

For studying the biochemical, serological and other properties of the isolated cultures, in all cases we used the third generation in nutrient media.

On the first day of observation the strains under study fermented, with the formation of acid without gas, glucose, mannitol, maltose, and incompletely rhamnose. On the second to the fourth days, and later in some strains, acid formation was recorded in litmus milk, glycerin, and lactose. Some of the strains caused a weak splitting up of saccharose (18 strains out of 44). Upon checking the biochemical properties on the medium suggested by L. A. Timofeyeva, in the first two days there was noted a change of coloration which is characteristic for P. pestis: Column of the medium was orange, and the slanted part - blue. Starting with the third day a change was recorded in the color of the tapered surface, which indicated the fermentation of lactose by the strains. It

must be noted that when studying the fermentation activity of the strains on carbohydrates the appropriate controls were set up -- with the P. pestis EV vaccine strain and two strains of E. coli. One of the strains of E. coli -- No 11 (reference number), intensively fermented the entire collection of carbohydrates, with the exception of saccharose.

Not one of the strains formed indole, some gave off hydrogen sulfide. The test with urea was negacive in all cases (the observations were carried out for three days). The reducing capability in respect to methylene blue was checked in 22 strains, also with negative results. All the strains reduced nitrates to nitrites, that is, possessed a denitrifying capability.

It must be noted that when studying later generations of the strains, results were obtained which testified to the heterogeneity of their cellular composition. In particular the appearance was recorded of a different type of colonies -- chromogenic and achromogenic with smoothened out contours. There was a marked division of the population of two strains into rhamnose-positive and rhamnose-negative variants (the populations of the remaining strains were not investigated in this respect). In the strains which fermented saccharose a tendency was ascertained toward the loss of this feature.

The ability for growth on hungry media was tested in 27 strains. The investigation showed that on deficient-acid agar the stated strains do not grow following the standard seeding. On peptone deficient agar in the first two days, growth was noted from 1--4 dilutions, and in later periods (5 days) in 12 strains the growth of individual colonies was recorded up to the 10th dilution.

All the strains turned out to be sensitive to polyvalent plague and pseudotuberculosis bacteriophage. During titration according to Appelman the plague caused lysis in 10^{-7} and 10^{-8} power of dilution with a phage titer of 10^{-9}. The sensitivity to pseudotuberculosis phage was approximately the same in the strains studied (10^{-6}, 10^{-7}).

Serological properties were studied in 20 strains -- they were all agglutinated by plague serum up to a titer (1:1600).

By the method of diffusion in agar with the help of discs, a check was made of the sensitivity of 21 strains to antibiotics; Streptomycin, biomycin, levomycetin, and penicillin. All the strains turned out to be sensitive to streptomycin. Different results were obtained in respect to biomycin and levomycetin. Out of the 21 strains checked, 13 turned out to be highly sensitive to levomycetin, 6 were mildly sensitive, and 2 were resistant. Biomycin acted on 13 strains, and 8 strains were resistant. It is interesting to note that almost all the strains (19 out of 21) were sensitive to penicillin.

The virulence of the isolated cultures was checked on white mice, guinea pigs, and white rats.

On white mice 23 strains were titrated. In the majority of these, doses of 10 and 100 microbial bodies (m.t.) caused the death of part of the infected animals. With an increase of the infecting dose there was an increase in the percentage of mice which died. Absolutely lethal doses were: 10 m.t. in one strain, 100 m.t. in 5 strains, 1,000 m.t. in 13 strains, 10,000 m.t. in 3 strains, and 100,000 m.t. in one strain.

The virulence of 9 strains was studied on guinea pigs. The infections were carried out subcutaneously. Infection doses up to 1 billion m.t. were used. Of the entire number of test animals only one pig died from one billion m.t. This was in 11 days following infection, and P. pestis was seeded out from the regional lymph node. The rest of the animals remained healthy and were destroyed in 30 days. A bacteriological investigation of them produced a negative result.

The white rats turned out to be sensitive to the isolated strains. For these animals the Dcl equaled one billion m.t., but a significant number of them died from the administration of 10,000 and even 1,000 m.t.

In preliminary tests the immunogenic properties of 4 strains were studied. It was established that a single subcutaneous administration of 100 m.t., and larger doses of these strains protected guinea pigs from subsequent infection with 200 Dcl of the highly virulent P. pestis strain 261.

In this manner, the cultures of 1962, isolated in Armenia from common voles and their fleas, based on their main biological properties (relatively weak virulence, fermentation of rhamnose and glycerin) are identical on the whole to the strains isolated during the epizootic of 1958--1959 in northwestern Armenia.

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